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ATCC

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Bristol-Myers Squibb Co.
Attn: Robert J. Peach
P.O. Box 4000
Princeton, NJ 08543-4000

Deposited on Behalf of: Bristol-Myers Squibb Company

Identification Reference by Depositor:

Plasmid L104EA29YIg

Patent Deposit Designation

PTA-2104

The deposit was accompanied by: __ a scientific description a proposed taxonomic description indicated above.

The deposit was received June 20, 2000 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 28, 2000. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara E. Coupé, Administrator, Patent Depository

Date: June 30, 2000

cc: Audrey F. Sher (Ref. Docket D0028)

BPA/9

LEA29Y

- Fusion protein of mutated CTLA4-Ig and human IgG1 Fc domain
- Mutations at position 29 and 106 confer
  - Comparable binding to B7-1
  - 10-fold higher avidity to B7-2 than parent CTLA4-Ig molecule

LEA29Y structure

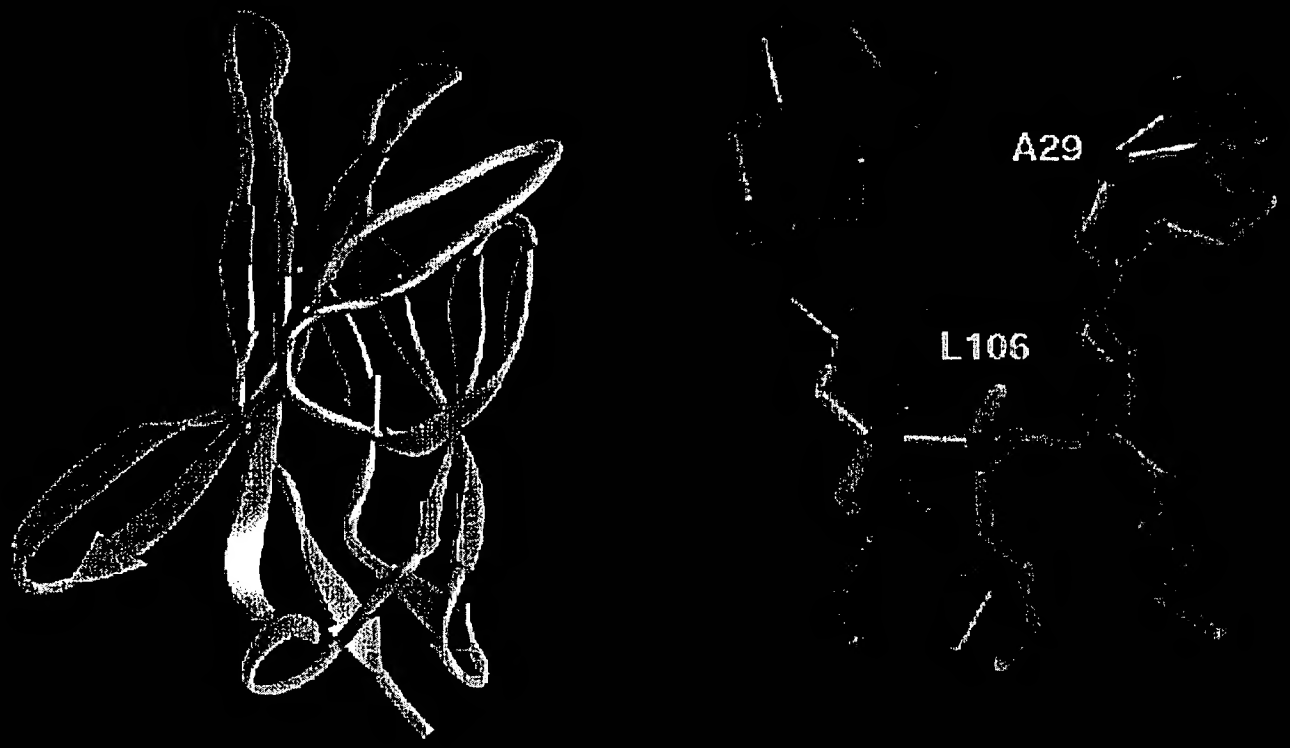
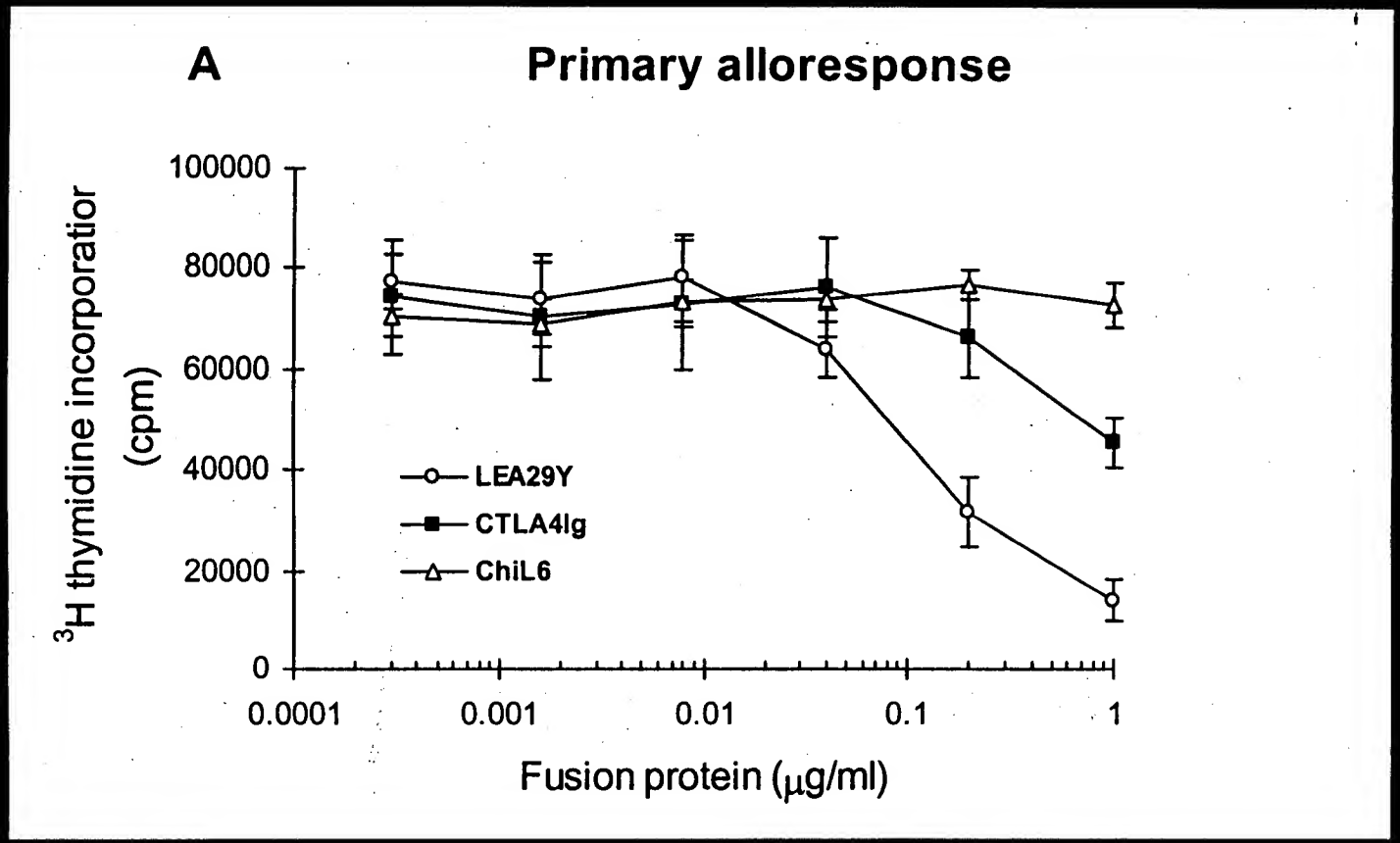


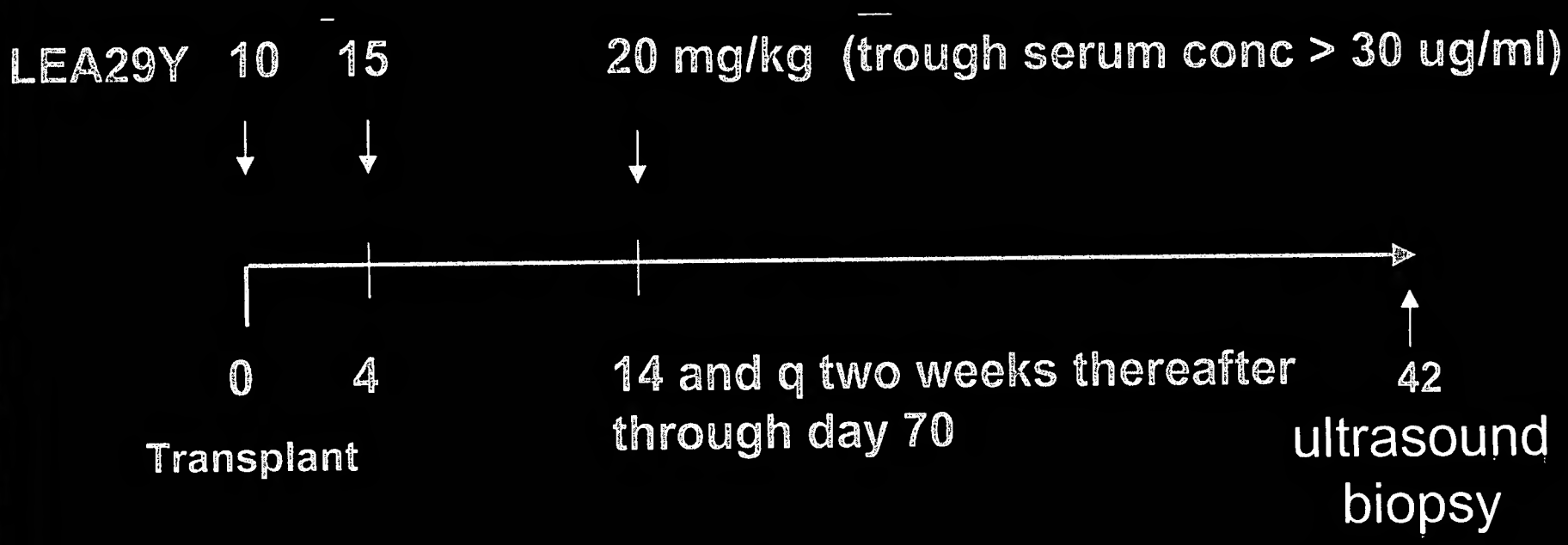
Figure 613. (left) A ribbon diagram of the CTLA-4 is shown with the CDR1- and CDR3-analogous loops colored green and magenta, respectively. (right) An expansion of the CDR1- and CDR3-analogous loops indicating the location of the avidity-enhancing mutations, L106 and A129.

Slide 2


A
Primary alloresponse
^{3}H thymidine incorporation (cpm)
100000
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
Fusion protein (μg/ml)
LEA29Y
CTLA4Ig
ChiL6

Slide 3

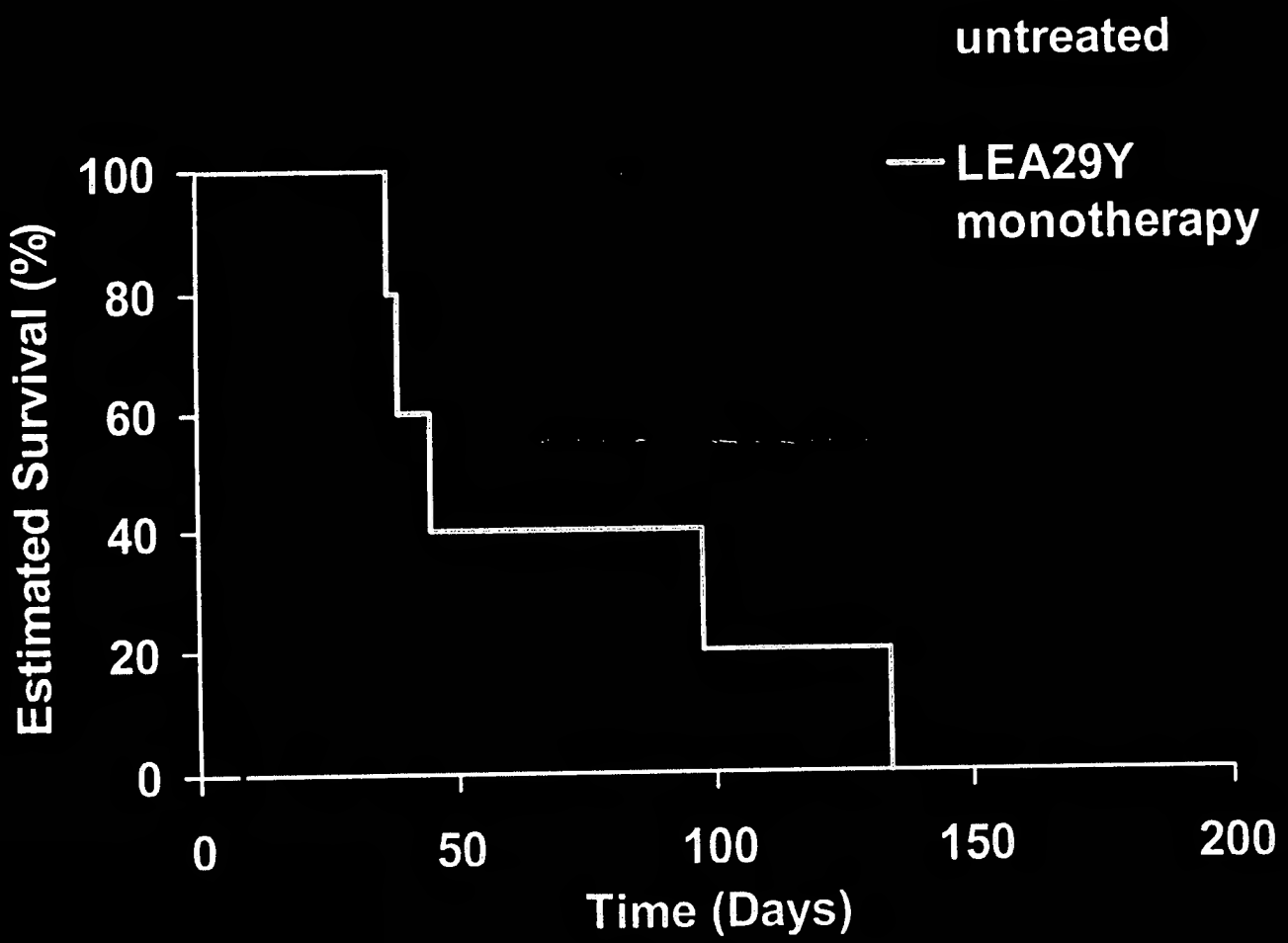
Effect of LEA29Y monotherapy on Renal allograft survival in macaques



(no treatment of acute rejection episodes)

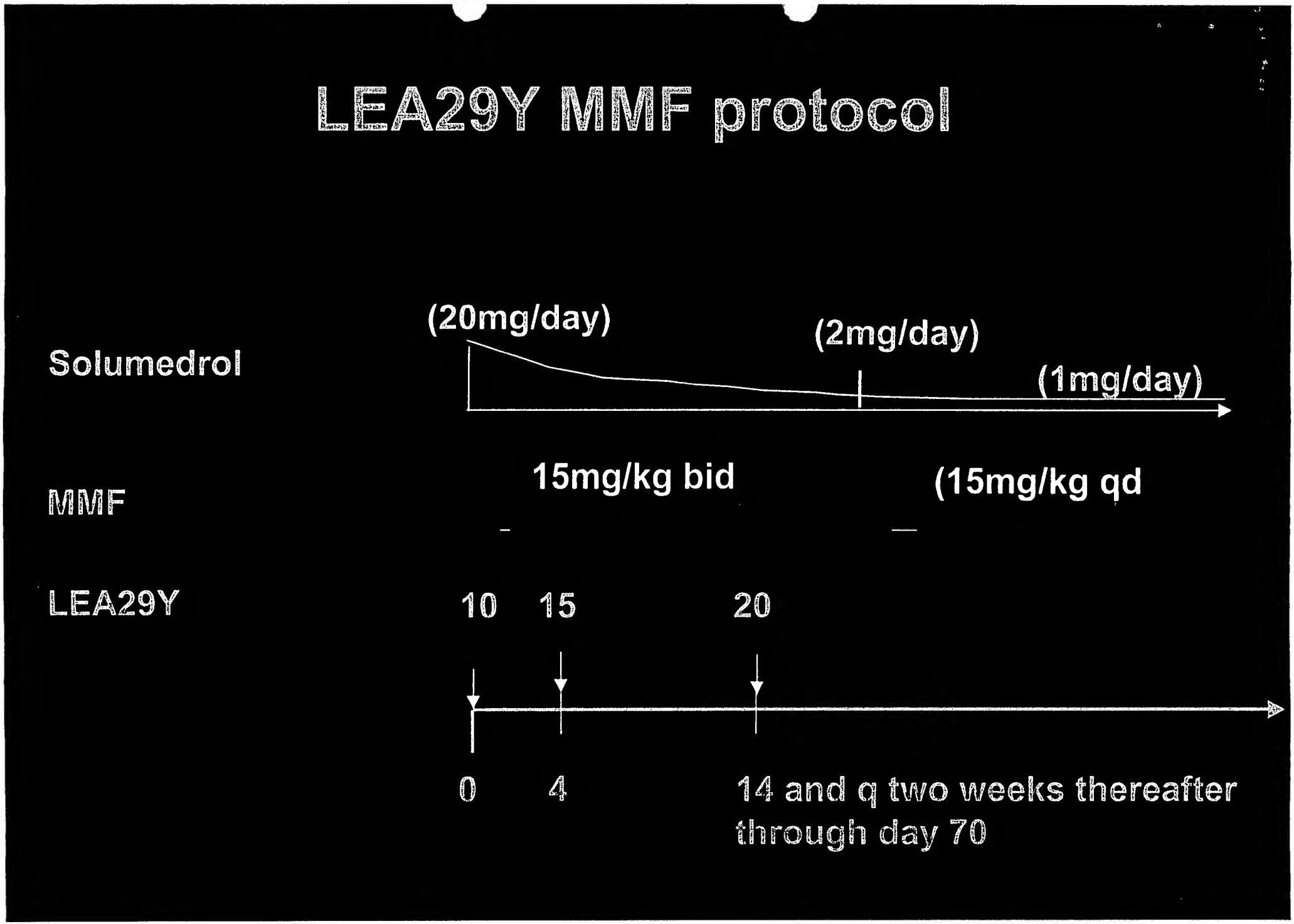
Slide 4

LEA29Y monotherapy prolongs allograft survival in rhesus macaques



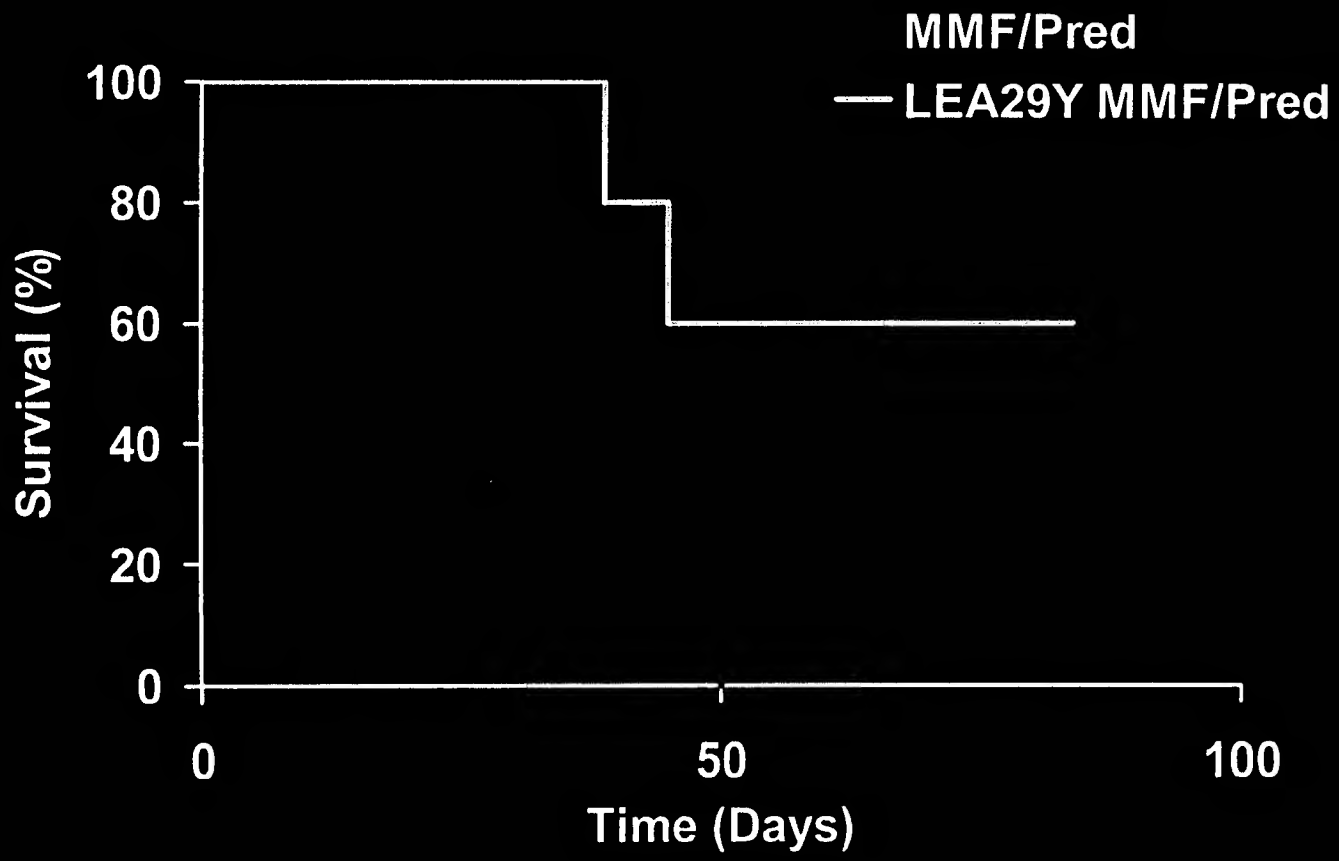
Slide 5

LEA29Y MMF protocol



Slide 6

Effects of LEA29Y, MMF and prednisone on renal allograft survival in rhesus macaques

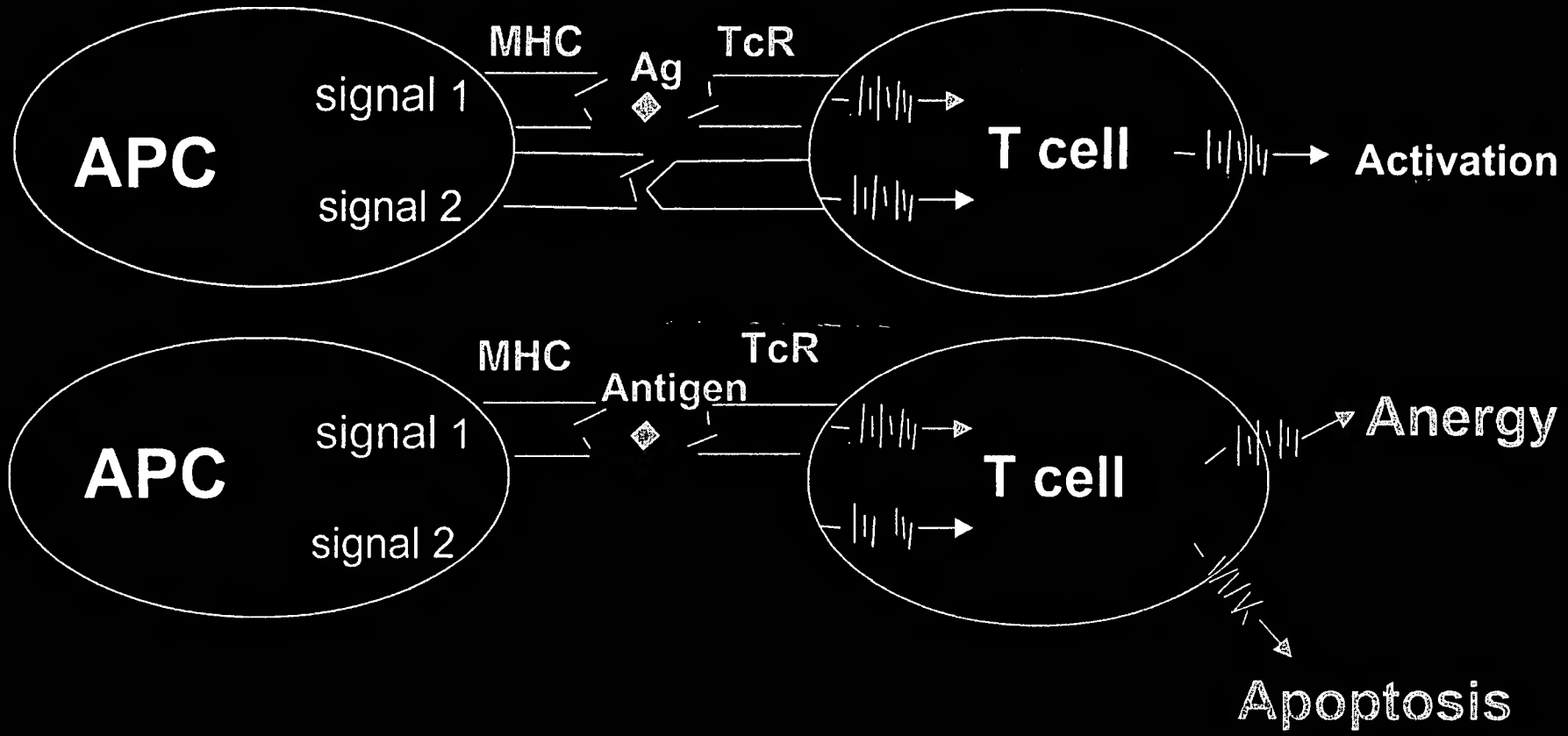


Slide 7

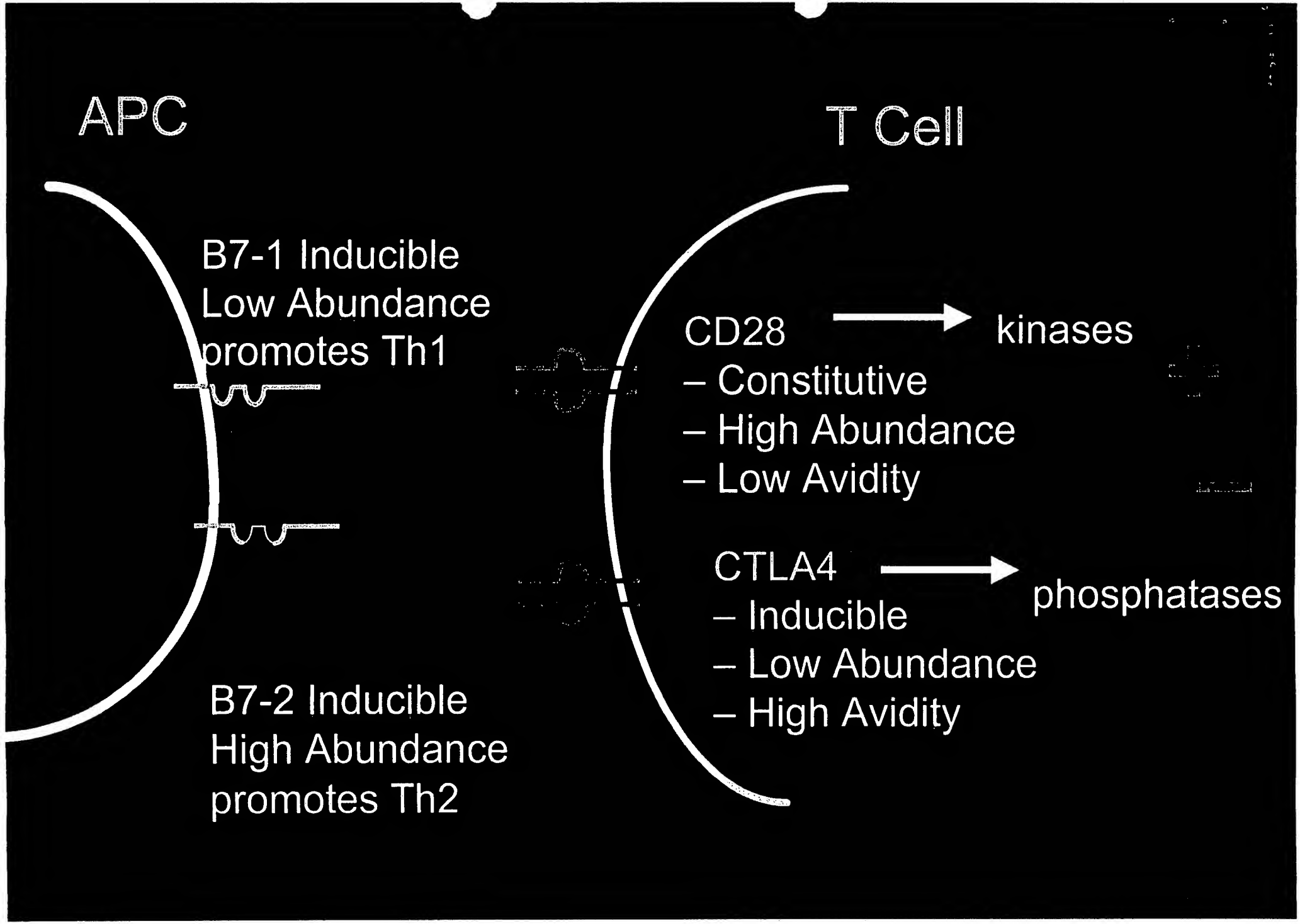
CD28 Summary

- Manipulation of the CD28 pathway inhibits allograft rejection and inhibits anti-donor Abs
- Second generation molecules (LEA29Y) may offer enhanced efficacy
- Addition of adjunctive agents will be necessary
  - Interactions with conventional immunosuppressants remains an unresolved issue
  - however addition of MMF/pred enhanced the efficacy of LEA29Y

Two signal model of T cell activation



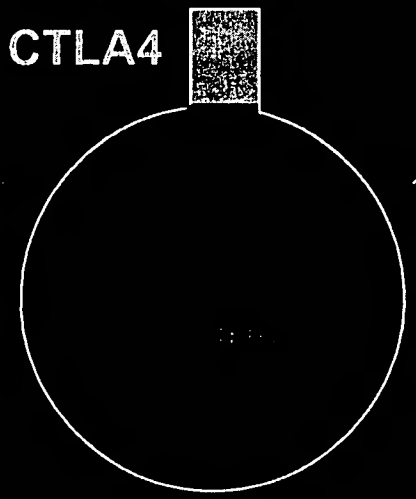
Slide 9


APC
T Cell
B7-1 Inducible
Low Abundance
promotes Th1
B7-2 Inducible
High Abundance
promotes Th2
CD28
kinases
– Constitutive
– High Abundance
– Low Avidity
CTLA4
phosphatases
– Inducible
– Low Abundance
– High Avidity

Slide 10

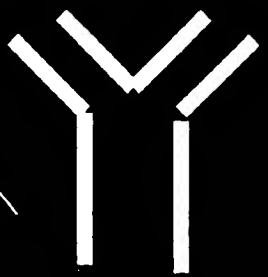
CTLA4 – Ig

CTLA4 human Ig



CTLA4

High Avidity
B7-1 > B7-2
- mouse
- rat
- rhesus
- human



Ig

Slide 11

CD28 blockade: Lessons from murine models

- **Agents**
  - **CTLA4-Ig- a potent inhibitor of T cell responses *in vitro* and *in vivo***
    - » Lenschow, et al. *Science* 1992
    - » Turka, et al. *PNAS* 1992
  - **Anti B7 monoclonal antibodies**- blockade of B7-1 and B7-2 required
    - » **Lenschow et al 1995. *Transplantation* 60:1171.**
    - » **Pearson et al 1997. *Transplantation* 63:1463.**
- **Timing of blockade may influence efficacy/outcome**
  - » Lin et al, *J Exp Med* 1993
  - » Sayegh et al, *J Exp Med* 1995
- **Adjuncts enhance efficacy**
  - **DST**
  - **Anti-CD40L**
  - **Anti-CD4**

CD28 blockade: Lessons from murine models

- Mechanism of action
  - Cellular effects
    - » inhibition of T cell activation/expansion
    - » Anergy
    - » Immune deviation
    - » Peripheral deletion
  - Molecular mechanisms
    - » Preferential CTLA4 signaling
    - » altered balance of regulators of apoptosis
- Interactions with "conventional" immunosuppressive agents may be important
  - Calcineurin inhibitors and corticosteroids
  - Rapamycin

CD28 blockade in primates

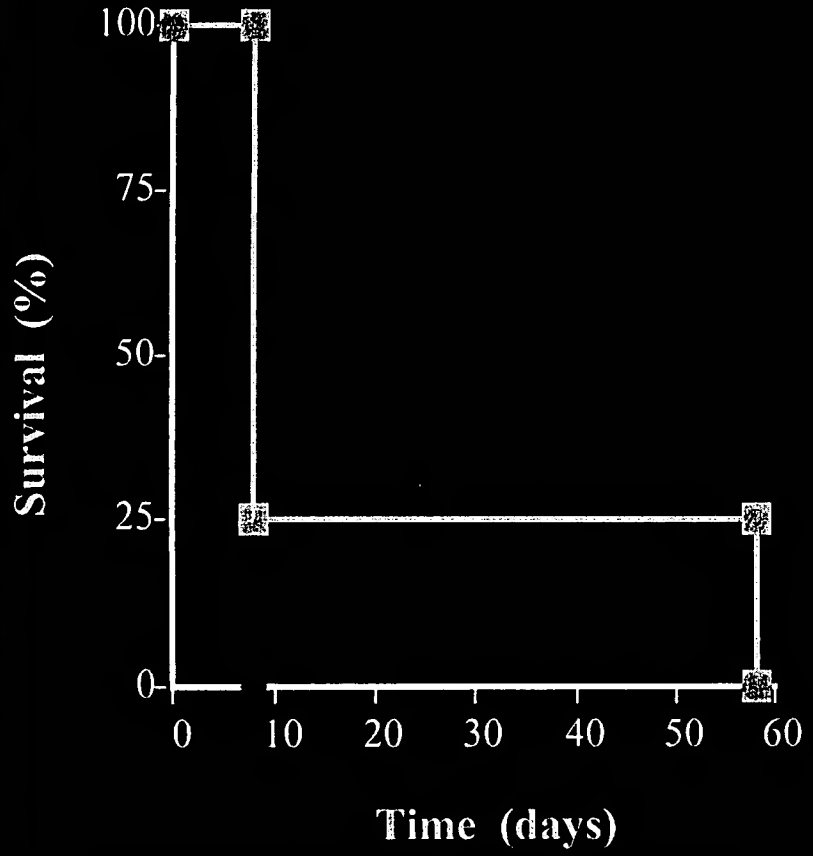
- CTLA4-Ig monotherapy has very modest effects on allograft survival and inhibits anti-donor antibody responses
  - Kirk, A.D et al 1997. PNAS 94,16:8789.
  - Levisetti et al 1997. JI 159:5187.
- Effect of anti-B71 and anti-B72 similar to CTLA4-Ig
  - Ossevoort et al 1999 Transplantation 68, 1010

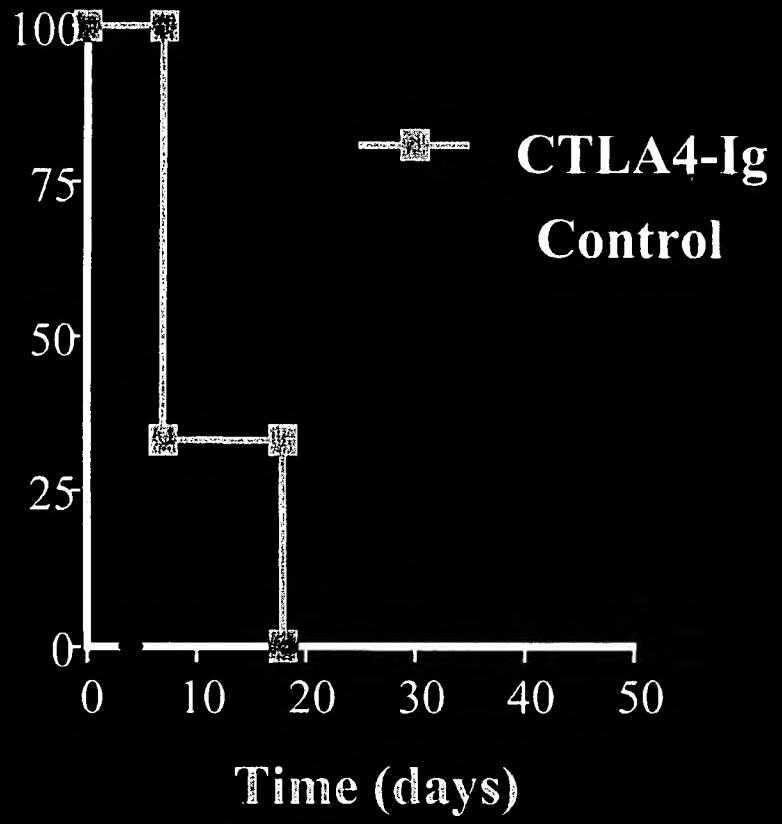
Effect of CTLA4-Ig monotherapy on Renal allograft survival in macaques

CTLA4-Ig (16 mg/kg) —

Slide 15

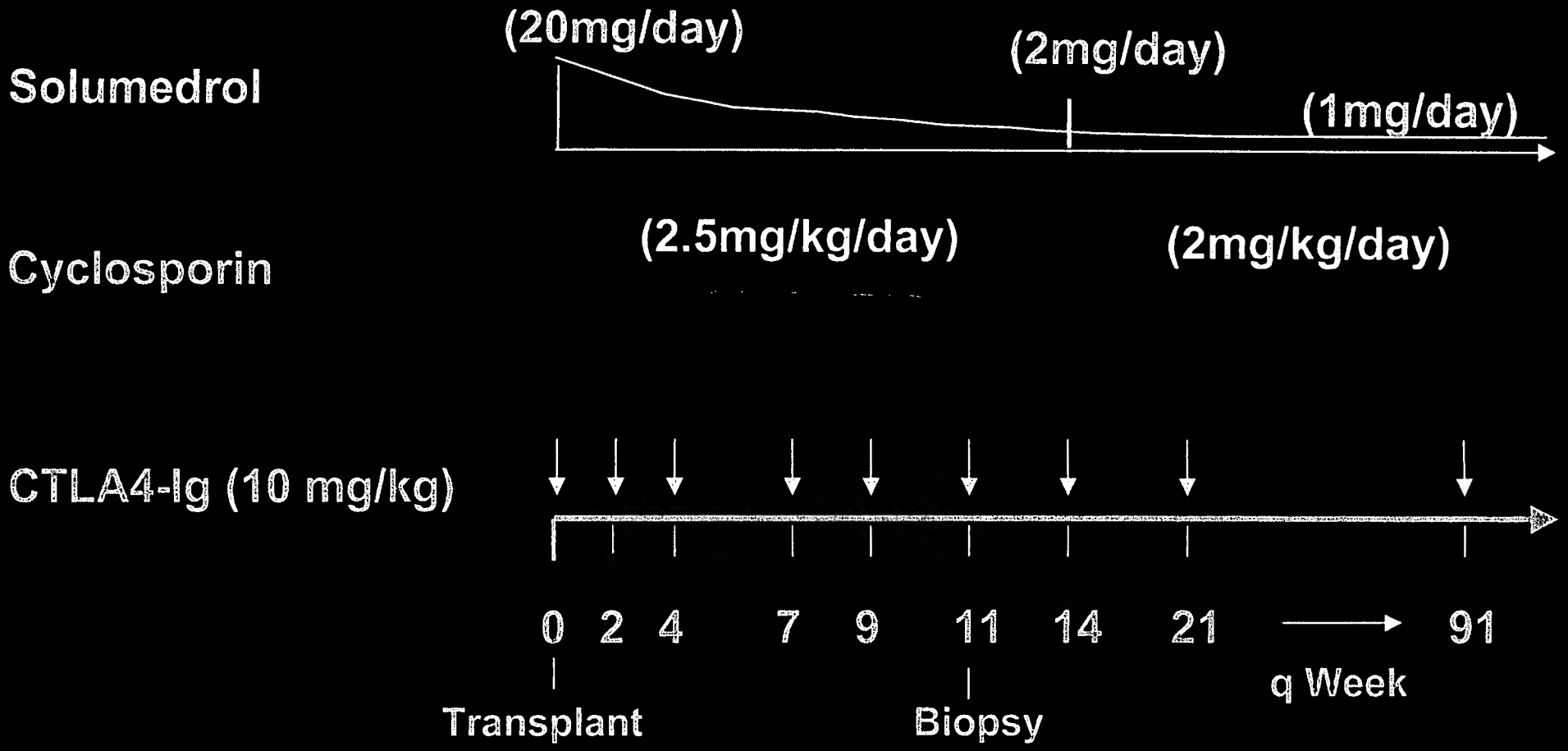
CTLA4-Ig monotherapy minimally prolongs renal allograft survival





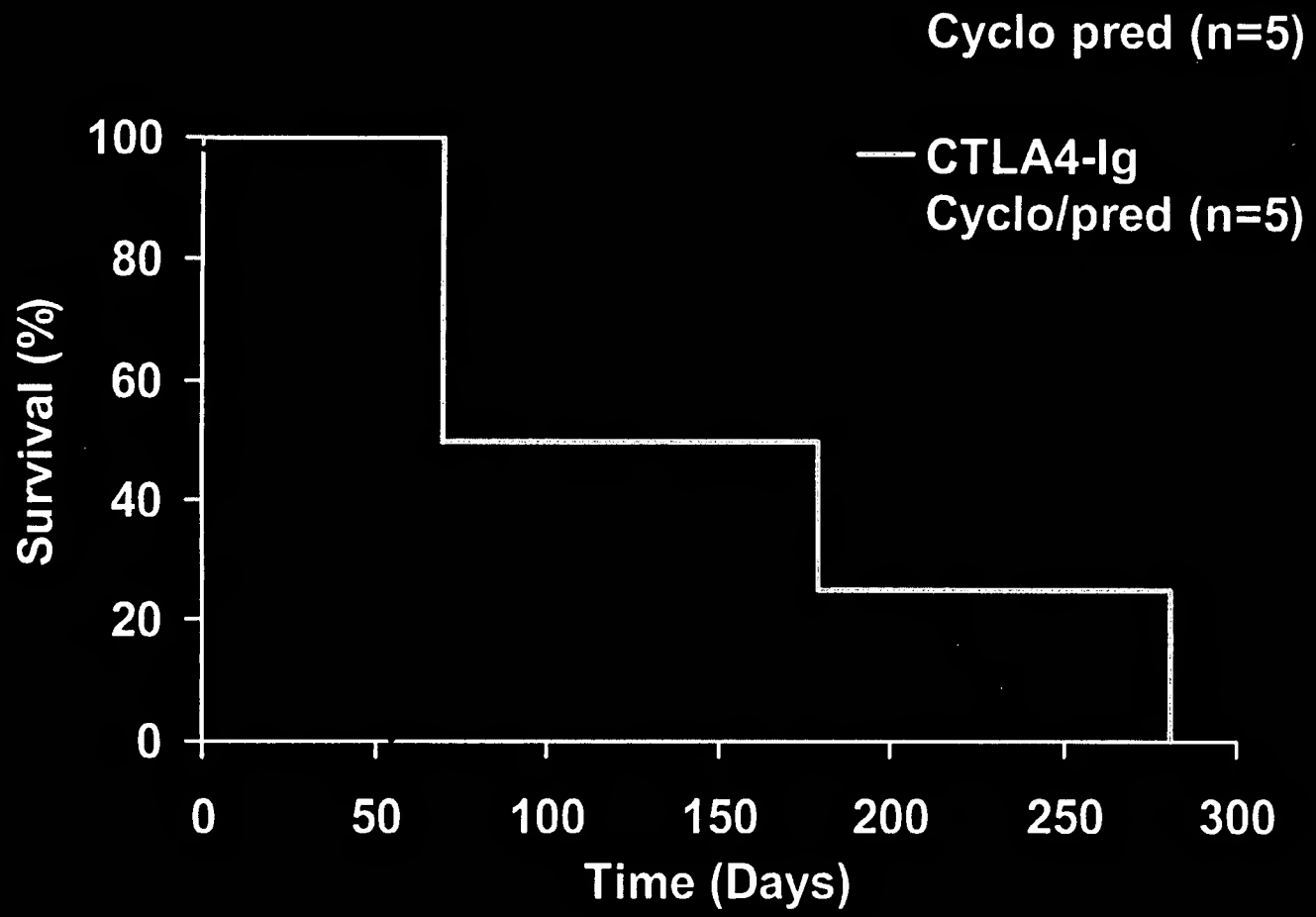
Slide 16

CTLA4-Ig and conventional immunosuppression



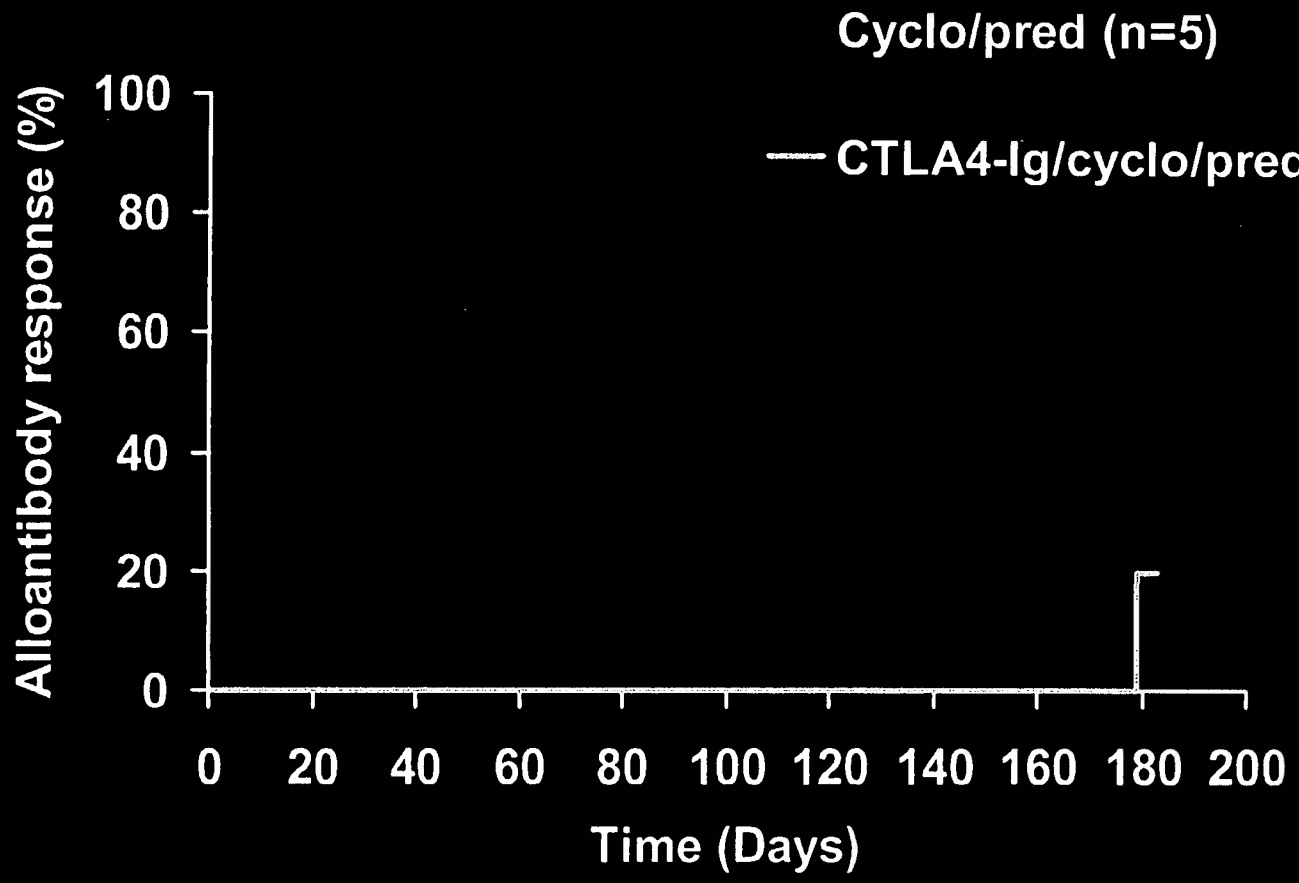
Slide 17

CTLA4-Ig prolongs allograft survival when given with conventional immunosuppression



Slide 18

CTLA4-Ig inhibits anti-donor antibody responses



Slide 19

Summary

- CTLA4-Ig is well-tolerated and immunosuppressive in non-human primates
- CTLA4-Ig inhibits anti-donor Ab responses
- immunosuppressive effects of CTLA4-Ig and cyclosporin are additive in the Rhesus renal allograft model